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Galactosamine Hepatitis, Endotoxemia, and Lactulose

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Studies by Liehr et al. suggest that endotoxins are important in the pathogenesis of galactosamine hepatitis (Gal-N hepatitis) in rats. Lactulose (9.1 gm per kg per day) prevents hepatic lesions induced by Gal-N; an antiendotoxin effect of lactulose is postulated. However, commercial preparations of lactulose are contaminated with galactose, which shows a competitive action to Gal-N. To analyze the effect of galactose, male Wistar rats were pretreated with lactulose (Duphalac®, 9.1 gm per kg per day) and given Gal-N (375 mg per kg i.p.). After 24 hr, serum was analyzed for glutamic pyruvate transaminase, glutamate dehydrogenase, and sorbitol dehydrogenase activities. Pretreatment with Duphalac®, even 1 hr before Gal-N, abolished toxicity. Duphalac® contains 10 gm galactose per 100 ml. Galactose was given in a similar concentration and similar inhibition occurred. Pretreatment with purified lactulose (9.1 gm per kg for 5 days) diminished the effects of Gal-N but did not normalize enzyme concentrations. Because small doses of galactose (80 and 300 mg per kg) showed similar inhibitory effects, we conclude that the protective effect of commercial lactulose preparations is mainly due to galactose contamination and not to an antiendotoxin effect.

The role of intestinal endotoxins in hepatic disease is conflictive. In severe viral hepatitis, more endotoxemia has been found than in moderate cases (1-4); however, it is uncertain if the phenomenon is primary or secondary. Galactosamine hepatitis (Gal-N hepatitis) provides an opportunity to study the postulated effect of endotoxins on the liver as proposed by Liehr et al. (5-8). Circumstantial evidence has been put forward in support of the postulate that intestinal endotoxins brought into the liver by portal route contribute to the pathogenesis of this form of hepatitis. Colectomy before Gal-N administration protects rats from Gal-N and reduces endotoxemia. Surgical trauma and other injury act similarly and may be explained by appearance of α-macro-fetoprotein (α_MFP), an acute phase protein, which has inflammatory inhibiting properties (9, 10). Purified α_MFP given before Gal-N administration protects the liver (11). A strong argument supporting the endotoxin hypothesis is the finding by Liehr et al. (12, 13) that lactulose given orally for 5 days before Gal-N administration prevents liver damage. This has been explained by an antiendotoxin effect of lactulose.

We repeated these studies taking into account the fact that galactose, an impurity of many lactulose preparations, may influence the final result.

MATERIALS AND METHODS

Male Wistar rats (TNO-Zeist, The Netherlands) weighing 250 to 300 gm were used. Lactulose was a commercial preparation (Duphalac®, Philips-Duphar, Weesp, The Netherlands) containing 67 gm lactulose per 0 ml. Lactulose (9.1 gm per kg) was administered by stomach tube each day for 5 days, or as a single dose 1 hr before Gal-N administration. Because commercial lactulose (Duphalac®) contains 10% galactose, the experiments were repeated substituting galactose for lactulose (0.9 gm per kg) equivalent to that in commercial lactulose (Duphalac®). Purified lactulose (96.6%) (Philips-Duphar) was used in a similar dose for 5 days (9.1 gm per kg). One hour after administration of lactulose by stomach tube, galactose was estimated in portal or peripheral venous blood. Galactosamine was given in a dose of 375 mg per kg i.p. 1 hr after the last dose of lactulose or galactose. Twenty-four hours after Gal-N administration, serum activity of glutamic pyruvate transaminase (GPT), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GLDH) was measured. Enzyme and galactose estimations were made with commercially available tests (Boehringer Mannheim, Mannheim, Germany).

Endotoxin (LPS) activity was measured by the method of Thomas et al. (14) with slight modifications. As a reference preparation, LPS Novo-Pyrexal (S abortus equi

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lipopolysaccharide Na form, Pyrotel Diagnostics, Oberursel, Germany) was used; this preparation is well defined (15). LPS-activated proenzyme in limulus lysate was measured with the chromogenic substrate S2222 [Benzoyl-Ile-Glu-(γ-oR)-Gly-Arg-*p*-nitroaniline·HCl, where R is 50% H and 50% CH_3, Kabi-Vitrum, Amsterdam, The Netherlands].

Statistical comparison of data between groups was performed with Student's t test.

RESULTS

After Duphalac® administration for 5 days or in one dose 1 hr before Gal-N administration, rats were resistant to Gal-N; Duphalac® given 1 hr before Gal-N had the same protection (Table 1).

Duphalac® contained 10.5 gm galactose per 100 ml. Galactose simultaneously appeared in portal and peripheral blood 1 hr after Duphalac® administration (Figure 1). Portal blood contained significantly higher concentrations of galactose, indicating the intestinal origin of this monosaccharide ($p < 0.01$, $n = 6$).

The effects of orally administered galactose on Gal-N hepatitis are presented in Table 2. We used a dose of galactose comparable to the amount in commercial lactulose, i.e., 0.9 gm per kg. Galactose in amounts found in commercial lactulose (Duphalac®) prevented Gal-N hepatitis. Rats were treated with the same dose of purified lactulose as Duphalac® (9.1 gm per kg a day) for 5 days (Table 3). All parameters showed a reduction in liver damage (SDH, $p < 0.01$; GPT, $p < 0.005$; GLHD, $p < 0.001$).

To exclude the possibility that lactulose is hydrolyzed by intestinal bacteria providing galactose to the portal tract, we estimated plasma portal venous galactose levels after 5 days of administrating purified lactulose. Galactose concentrations did not exceed normal values 1 hr after the last lactulose administration (0.02 to 0.27 mmole per liter).

Purified lactulose is, to a minor degree, contaminated with small quantities of galactose. We found 0.75% in different samples. Consequently, we administered low doses of galactose: 80 mg per kg for 5 days and 300 mg per kg for 3 days. The results are presented in Table 3. The protective effect of 80 mg galactose is not significantly different from the effect of purified lactulose (only GLDH reaches a p value of $0.01 > p < 0.02$).

To exclude an antiendotoxin effect of galactose, we compared calibration curves of LPS soluted in distilled LPS-free water with and without galactose (Figure 2A).

Simultaneously, we tested lactulose (Duphalac®) against the same LPS concentrations (Figure 2B). The ratio between concentrations of galactose (10 μg per liter) or lactulose (67 μg per liter) and LPS concentration (100 ng per liter) is the same as in the experiments of Liehr which prevented limulus lysate gel formation. Neither

TABLE 1. EFFECT OF GALACTOSAMINE ADMINISTRATION ON SERUM GPT, SDH, AND GLDH IN CONTROL AND DUPHALAC®-TREATED RATS

Treatment	No. of rats	GPT (units/liter)	SDH (units/liter)	GLDH (units/liter)
Normal values no Gal-N	6	24 ± 2	9 ± 3	10 ± 3
Gal-N without pretreatment	12	2,117 ± 414	550 ± 117	185 ± 37
Gal-N after 5 days of Duphalac® administration	6	23 ± 2[a]	9 ± 3[b]	20 ± 2[a]
Gal-N after a single gift of Duphalac®	6	24 ± 4[a]	11 ± 3[b]	22 ± 2[a]

Values are expressed as mean ± S.E. Gal-N (375 mg/kg i.p.) was administered in the treated groups 1 hr after the last gift of Duphalac® (9.1 gm/kg). All animals were killed 24 hr after Gal-N administration.

[a] $p < 0.005$ compared with Gal-N without pretreatment.

[b] $p < 0.01$ compared with Gal-N without pretreatment.

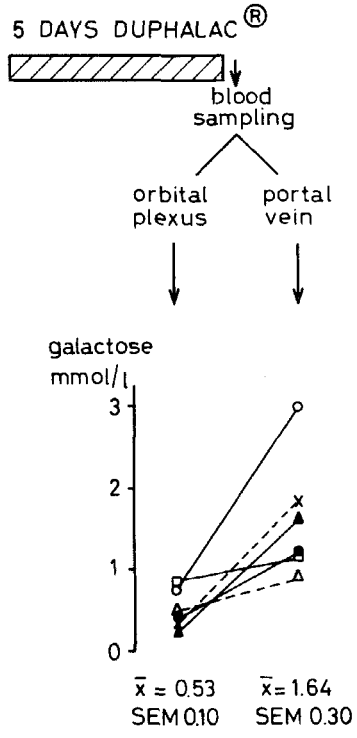

FIG. 1. Galactose concentrations (mmoles per liter) in peripheral and portal blood 1 hr after administration of Duphalac®.

TABLE 2. EFFECT OF GALACTOSAMINE ADMINISTRATION ON SERUM GPT, SDH, AND GLDH IN CONTROL AND GALACTOSE-TREATED RATS

Treatment	No. of rats	Galactose[a] (mmoles/liter)	GPT (units/liter)	SDH (units/liter)	GLDH (units/liter)
Gal-N without pretreatment	12	—	2,117 ± 414	550 ± 117	185 ± 37
Gal-N after 5 days of galactose administration	6	3.71 ± 0.26	23 ± 3[b]	18 ± 3[c]	16 ± 3[b]
Gal-N after a single gift of galactose	6	2.27 ± 0.34	20 ± 4[b]	9 ± 3[c]	10 ± 3[b]

Values are expressed as mean ± S.E. Gal-N (375 mg/kg i.p.) was administered in the treated group 1 hr after the last gift of galactose (0.9 gm/kg). All animals were killed 24 hr after Gal-N administration.

[a] Galactose concentration in orbital plexus blood 1 hr after galactose administration (0.9 gm/kg).

[b] $p < 0.005$ compared with Gal-N without pretreatment.

[c] $p < 0.01$ compared with Gal-N without pretreatment.

TABLE 3. EFFECT OF GALACTOSAMINE ADMINISTRATION ON SERUM GPT, SDH, AND GLDH IN CONTROL RATS AND RATS TREATED WITH PURIFIED LACTULOSE AND GALACTOSE

Treatment	No. of rats	GPT (units/liter)	SDH (units/liter)	GLDH (units/liter)
Gal-N without pretreatment	6	2,968 ± 577	883 ± 255	146 ± 9
Gal-N after 5 days of purified lactulose administration	6	425 ± 180[a]	129 ± 45[b]	48 ± 11[c]
Gal-N after 5 days of galactose administration (80 mg/kg)	4	961 ± 300[d]	190 ± 48[d]	122 ± 26[e]
Gal-N after 3 days of galactose administration (300 mg/kg)	6	295 ± 83	36 ± 12	52 ± 19

Values are expressed as mean ± S.E. Gal-N (375 mg/kg i.p.) was administered 1 hr after the last gift of purified lactulose (9.1 gm/kg) or galactose. Galactose (80 mg/kg) equals the amount of galactose present in the purified lactulose preparation. All animals were killed 24 hr after Gal-N administration.

[a] $p < 0.005$ compared with Gal-N without pretreatment.
[b] $p < 0.01$ compared with Gal-N without pretreatment.
[c] $p < 0.001$ compared with Gal-N without pretreatment.
[d] $p < 0.05$ compared with Gal-N without pretreatment.
[e] $p < 0.02$ compared with the group pretreated with purified lactulose.

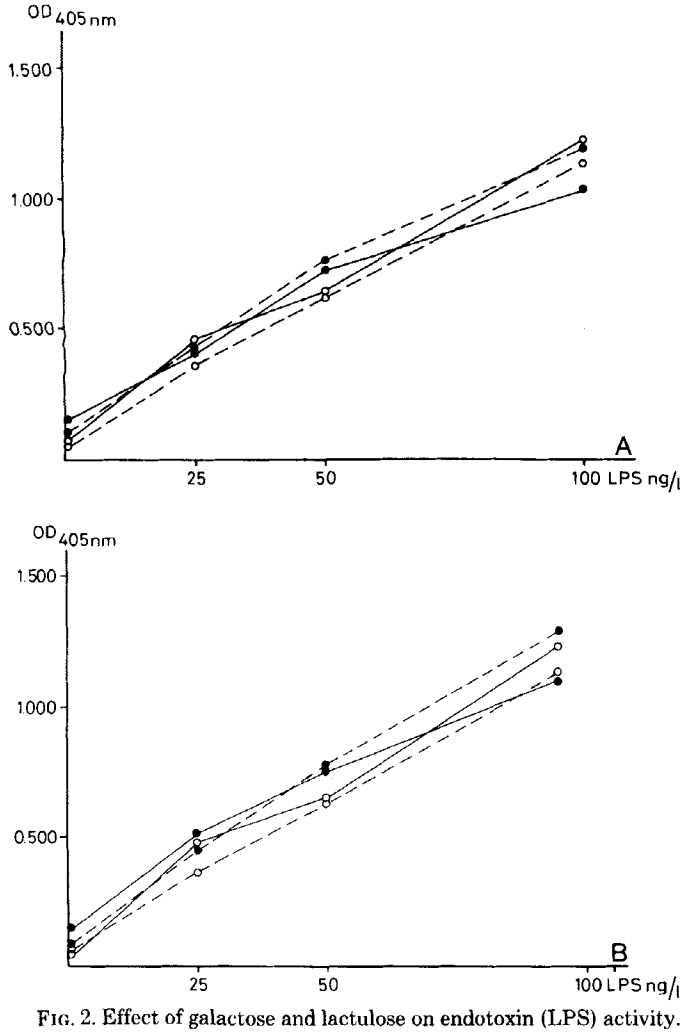

FIG. 2. Effect of galactose and lactulose on endotoxin (LPS) activity. Calibration curves of an LPS solution with (-●-) and without -○-) addition of galactose or lactulose (Duphalac®) were compared. Measurements were made directly (——) and after 24 hr of incubation at 37°C (- - -). On the *abscissa*, LPS concentrations; on the *ordinate*, optical density at 405 nm. (A) Addition of galactose, concentration 10 μg per liter (-●-). (B) Addition of lactulose 67 μg per liter (-●-).

immediately nor after 24 hr incubation at 37°C was LPS activity inhibited by galactose or lactulose. The same lack of inhibition was found with galactose concentrations up to 10 mg per ml and lactulose concentrations up to 67 mg per ml.

Liver sections from rats 24 hr after Gal-N administration showed diffuse hepatocellular necrosis, inflammatory infiltration, and lobular disarray (Figure 3A). Pretreatment with galactose or Duphalac® resulted in normal lobular structure with slight Kupffer cell hyperplasia and no inflammatory infiltration (Figure 3B). Rats pretreated with purified lactulose showed periportal infiltration and reduced liver cell necrosis (Figure 3C) than did galactosamine controls.

DISCUSSION

Commercial preparations of lactulose are contaminated with galactose. Our preparation contained 10.5 gm per 100 ml which completely protects against the effects of Gal-N on the liver. Galactose protects against the primary biochemical lesion in Gal-N hepatitis (16) which involves the uptake of Gal-N into the liver and its metabolism to Gal-N-1-phosphate, uridine-diphosphate-Gal-N (UDP Gal-N), and UDP-glucosamine.

The metabolism of Gal-N depletes several uracil nucleotides including UDP-galactose and UDP-glucose (17) which impairs glycoprotein synthesis and alters the composition of cell membranes. Cellular damage leads to inflammation resulting in a histological and biochemical picture closely resembling viral hepatitis.

Administration of galactose up to 30 min after Gal-N challenge inhibits the uptake of Gal-N into the liver and prevents UDP-glucose and UDP-galactose deficiency. Adequate protection begins when galactose is given in equimolar quantities with Gal-N (16). Reutter et al. (16) postulated that a single carrier exists in the liver cell membrane for both sugars. In addition, Gal-N is metabolized by enzymes of the galactose pathway, such as galactokinase. The affinity of galactokinase for galactose is 10 times higher than for Gal-N (K_m 0.2 and 2.0 mM, respectively). Chemically purified lactulose also protects; however, this preparation also contained 0.75% galactose. In experiments with purified lactulose, 72 mg per kg galactose were administered; enzyme activities did not differ from those with lactulose alone for GPT and SDH (Table 3) when rats were given 80 mg per kg galactose; only GLDH was higher in experiments with galactose ($p < 0.02$).

In the rat, small intestine aerobic and anaerobic Lactobacilli and coliforms are present which can metabolize lactulose; it is not known whether hydrolysis, which releases galactose, occurs. After administration of purified lactulose, no significant elevation of galactose appeared in portal venous blood. Because of our special interest in α_MFP as an acute phase protein of the rat with strong, inflammatory inhibiting properties, we estimated the plasma level of this protein in all experiments. Lactulose, purified or not, did not elevate plasma α_MFP level. We conclude that the protective effect of commercial lactulose in Gal-N hepatitis is probably due to galactose and not to an antiendotoxin effect.

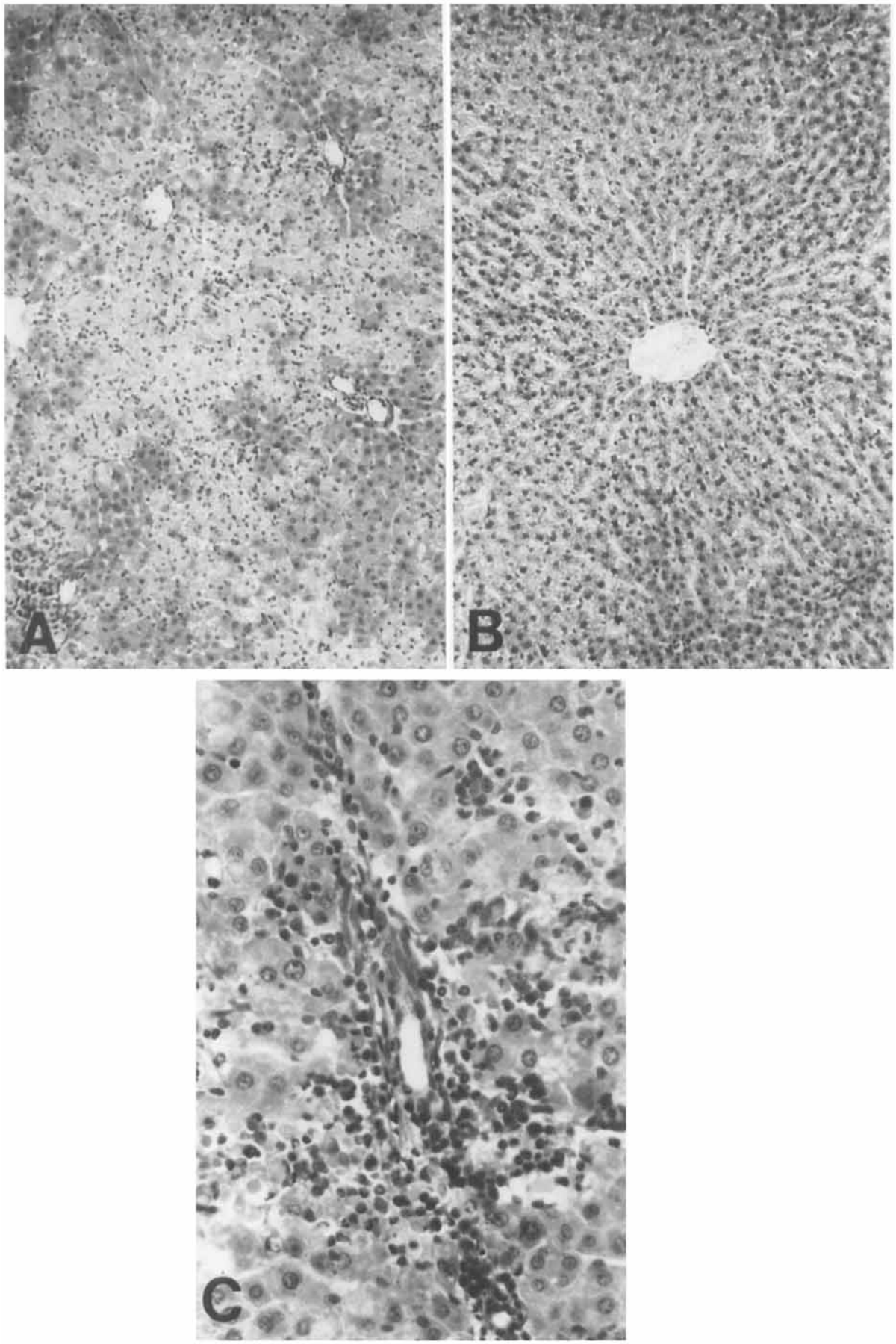

FIG. 3. Photomicrographs from galactosamine controls and rats pretreated with galactose or purified lactulose. (A) Liver section from Gal-N control showing necrosis of liver cells inflammatory infiltration and lobular disarray (H & E, × 140). (B) Liver section from a rat given 0.9 gm per kg galactose 1 hr before galactosamine administration showing normal array of liver cells and minimal infiltration (H & E, × 140). (C) Liver section from a rat pretreated with purified lactulose shows periportal infiltration (H & E, × 350).

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